Population Ecology of *Botrychium* (Moonworts)

Status Report on Minnesota *Botrychium* Permanent Plot Monitoring

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INTRODUCTION

The genus *Botrychium* (moonworts) is among the rarest fern genera in Minnesota. Plants are found in the western native prairies as well as the northern rich hardwoods. Several of the species in this genus are listed as endangered and have very limited distributions. *Botrychium gallicomontanum*, Frenchman's Bluff Moonwort, is known only from three sites worldwide in northwestern Minnesota. Other species that are rare in Minnesota include B. acuminatum, B. lunaria and B. pseudopinnatum. *Botrychium mormo*, no longer considered rare, has a limited distribution in the Great Lake States and has recently created conflict between conservation and timber harvest interests due to its limited distribution.

Botrychium subgenus *Botrychium* is found worldwide in a variety of habitats including grasslands and forests. This subgenus produces a small, single leaf (two to ten cm) which is divided into a once- or twice-pinnate sterile segment, known as the trophophore, and a twice- or tri-pinnate fertile segment, known as the sporophore. The plants generally produce one leaf annually although it is common for *Botrychium* to remain dormant underground producing no aboveground leaf in a given year. The underground rhizome is upright and short with mycorrhizal stem and roots and a single leaf-producing bud at the apex. The bud may contain up to five preformed leaves.

Relatively little of the life cycle is visible above ground. Plants produce spores that filter down into the soil and germinate in darkness. Following germination a subterranean achlorophyllous fleshy gametophyte is produced. The gametophyte produces gametangia and sexual reproduction occurs resulting in a subterranean juvenile sporophyte. It appears that it takes several years for this juvenile sporophyte to produce an apex and emerge above ground (Johnson-Groh, Farrar and Miller, 1998). Some species reproduce asexually via underground gemmae, small (0.5-1mm) propagule that can independently start a new plant once detached from the parent plant (Farrar and Johnson-Groh, 1990). Much about the underground aspects of the life cycle remains unknown.

The author began ecological studies of *Botrychium* in 1986 with the monitoring of prairie moonworts (*B. campestre*, *B. gallicomontanum* and *B. simplex*). These studies have resulted in reports and publications on the ecology and morphology of the species (Farrar and Johnson-Groh, 1986; Johnson-Groh, 1988; Farrar and Johnson-Groh, 1990; Farrar and Johnson-Groh, 1993; Johnson-Groh, 1995; Johnson-Groh, 1997), a new species description (Farrar and Johnson-Groh, 1991) and several papers currently in preparation.

The 1998 field season represented the twelfth year of monitoring *Botrychium* populations. This work is currently being analyzed in preparation for publication. The phenology project conducted in 1996-97 along with a paper modeling population extinction probabilities were presented at the annual meetings of the American Institute of Biological Sciences in August 1998 and currently are in preparation for publication. Johnson-Groh has initiated similar monitoring studies on western species of *Botrychium* in 1997-98 in Oregon, Washington and Alaska. These western projects are to investigate the population demographics, underground ecology and management effects of timber harvest and burning on populations.

This report is an update of the monitoring that has been conducted in Minnesota for 12 years on prairie moonworts (*B. campestre* and *B. gallicomontanum*), 7 years on *B. mormo* and 2 years for Lake Bronson *B. gallicomontanum* plants.

METHODS

Fifteen permanent *Botrychium* plots monitoring five different species have been established in nine sites in Minnesota. Plots have been monitored annually as follows: Each plot contains 5.7m2 in which each individual plant is marked by a numbered tag attached to an aluminum wire inserted into the ground two cm north of the plant. (Potential affects of the tags on plant growth have been tested and ruled out through comparative studies on "tagless" plots.) Each tag is checked for presence or absence of plants. Plants are measured and notes are recorded on the degree of development (just emerging, releasing spores, etc.) as well as disturbances such as herbivory or fire damage. New plants are tagged. This same procedure has been used on all plots in Minnesota (and on western species) allowing direct comparisons between species and species areas. Extreme caution is taken to avoid damaging plants and thereby affecting subsequent measurements.

Plots are monitored annually at approximately the same phenological stage of development. *B. campestre, B. simplex* and *B. gallicomontanum* are typically monitored the first week of June and *B. mormo* is monitored in late July or early August.

RESULTS

Botrychium campestre

Botrychium campestre is found primarily in the western portion of Minnesota in glacial till prairies. It has also been found in the southeastern portion of the state and most recently on mining spoils in the northern portion of the state. Outside of Minnesota *B. campestre* is widespread in the Great Lakes, Iowa and in the northern Great Plains.

Botrychium campestre has been monitored in four plots located in Hole-in-the-Mountain preserve, Lincoln County. This preserve, owned by The Nature Conservancy (TNC), contains many plants scattered throughout the area on the shoulders of the bluffs. *B. campestre* was originally discovered at this site in the mid 80's. Monitoring began in 1989 with the establishment of four plots in different burn units.

The overall average number of plants per plot peaked in 1990 with an average of 13 plants per plot (Figure 1). The lowest population numbers have occurred recently with an average of 3 plants per plot in 1998. Whereas the overall trend is down it is important to notice that individual plots vary independently. For example plot 15 peaked in 1990 with 13 plants and then lost all above ground plants in 1993 and 1994 to rebound in 1995 and 1996 with populations of 2 and 1 respectively. When plot 15 was rebounding in 1995, plot 16 had no aboveground plants, but rebounded the following year with its second largest population. Plot 17 has consistently supported the largest population averaging 12 plants annually.

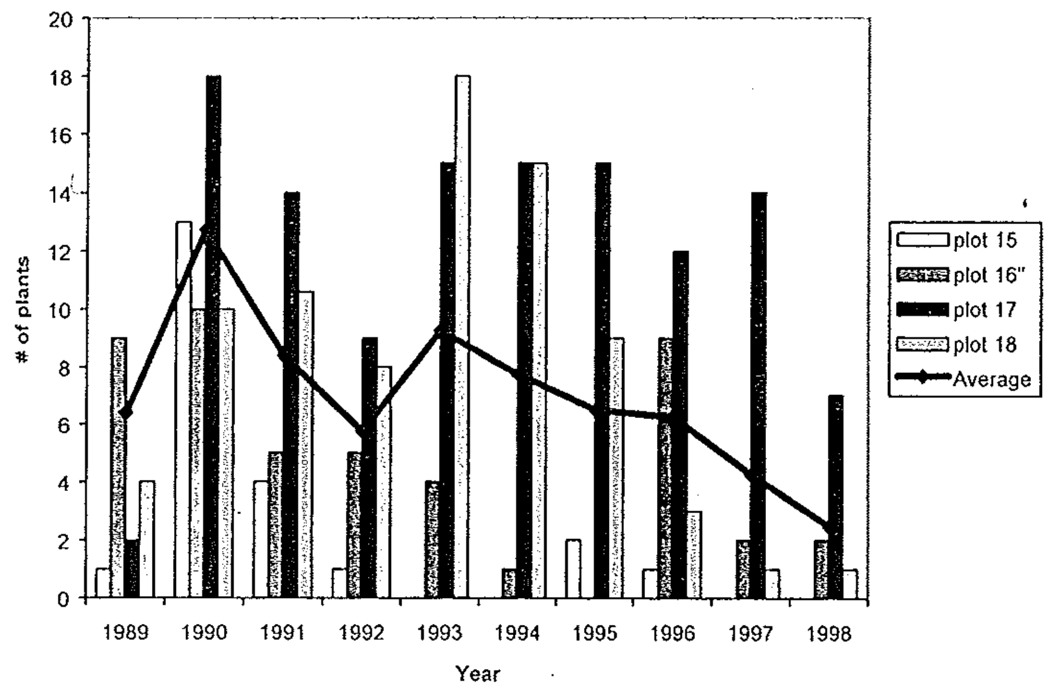

Figure 1. Population trends for *B. campestre* plots at Hole-in-the-Mountain, Lincoln County, Minnesota

B. gallicomontanum – Frenchman's Bluff

B. gallicomontanum is among the rarest species in Minnesota. It is endemic to Minnesota, restricted in its known occurrences to three sites in Northwestern Minnesota. The primary site, Frenchman's Bluff, in the ownership of the Minnesota Chapter of The Nature Conservancy, is a 42-acre preserve located in Norman County, Minnesota. The second site approximately 1 mile west of Frenchman's Bluff is privately owned. This site also contains all three prairie species (*B. gallicomontanum, B. campestre* and *B. simplex*) and has been moderately grazed, but supports a relatively undisturbed prairie. As one of only three known sites for *B. gallicomontanum* this site should be preserved. A third site, Lake Bronson, was discovered in 1997 and will be discussed separately below. Additional populations of *B. gallicomontanum* have not been found despite extensive searches conducted by the author over several years (Johnson-Groh 1988; 1995; 1997).

We have established six permanent plots at Frenchman's Bluff in two different burn units. Two plots were established in 1987 and four more were added in 1989. The overall average number of plants per plot peaked in 1997 with an average of 53 plants per plot (Figure 2). The highest populations have occurred recently with averages of 52, 53 and 47 plants per plot in 1996, 1997 and 1998 respectively. Unlike *B. campestre* the overall trend is an increase in plot population sizes. All plots with the exception of plot 5 have shown marked increases in populations.

The population in plot 5 dropped significantly in 1989 because of gopher excavation within the plot. Approximately half of the plot was buried in freshly excavated sediment and many tags were wholly covered. The population continued to decrease in 1990 and 1991 to a low of 8 plants. The population has since rebounded to 27 plants in 1998 which is within 6 plants of the population size in 1987 (33 plants).

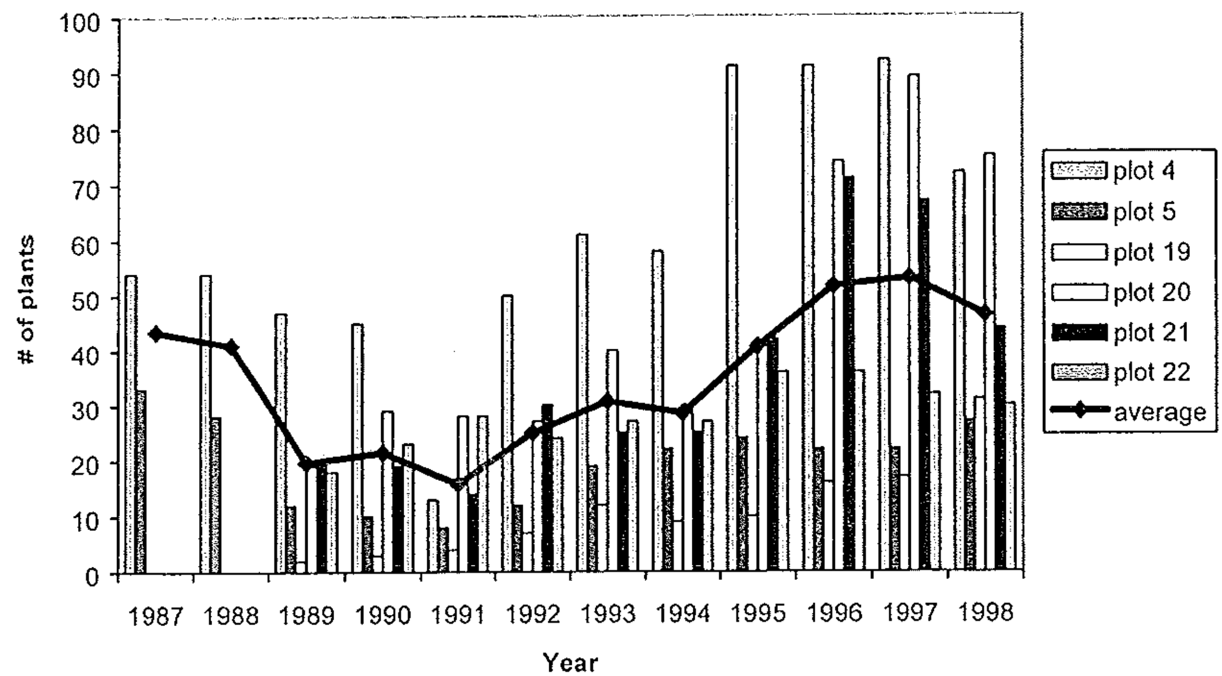

Figure 2. Population trends for *B. gallicomontanum* plots at Frenchman's Bluff Preserve, Norman County, MN.

The remarkable resiliency of *B. gallicomontanum* is further illustrated by the recovery of plot 19. Informal surveys done in 1986 revealed a dense population in the area of plot 19. Seventy-five plants (53 *B. gallicomontanum*, 22 *B. simplex*) were counted in a 10m^2 area. This plot was burned in 1987 and consequently no plot was established in this area until 1989 when one *B. gallicomontanum* was found. The population has slowly, but steadily increased to a high of 31 plants in 1998 (Figure 3). This slow, but predictable recovery can be attributed to the nature of the underground portion of the life cycle. It takes several years for the spores to germinate, produce underground gametophytes and eventually juvenile sporophytes. A very slow initial recovery followed by steady large increases is consistent with models of population growth for *Botrychium* (Johnson-Groh, Farrar, and Miller, 1998).

The density of *Botrychium* in plot 4 is the highest of the prairie *Botrychium* plots. Similar densities are found for *B. mormo* and western *Botrychium* species. I do not know why this area is so productive, but factors such as soil, moisture, mycorrhizae, and herbivory undoubtedly influence this density.

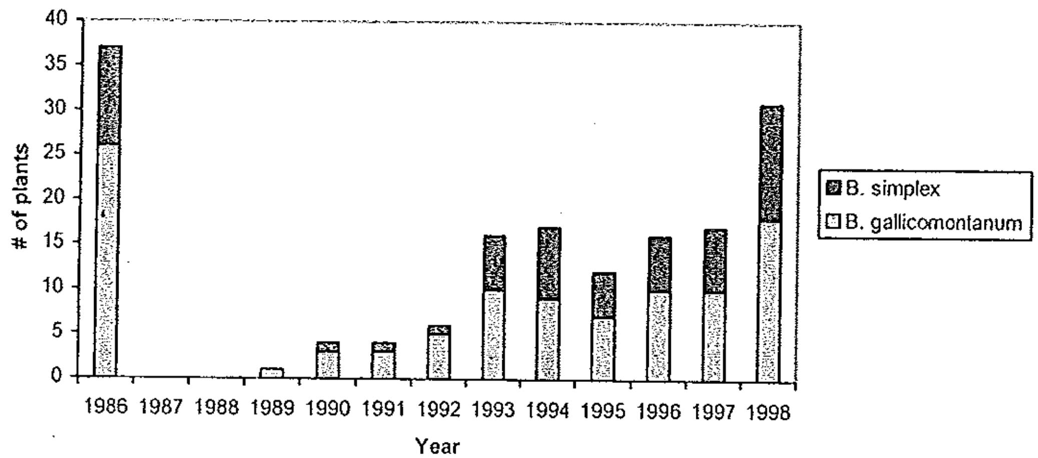

Figure 3. Plot 19 *B. gallicomontanum* population rebound following fire in 1987, Frenchman's Bluff Preserve, Norman County, MN.

B. gallicomontanum – Lake Bronson

A new population of *Botrychium gallicomontanum* was discovered at Lake Bronson State Park, Kittson County in June 1997 by the author. This population is in native prairie that had been extensively managed for controlling invasive weedy plants. The site had been burned in the fall of 1996 followed by machine seeding with prairie seeds in the spring of 1997. Finally the site was sprayed with the herbicide Roundup, on the 23rd of May 1997. We visited the site 13 days after the herbicide had been applied. Newly discovered moonworts were yellowed and deformed revealing obvious signs of damage. Two permanent plots were established in 1997 for studying the long-term effects of the herbicide.

Lake Bronson was revisited June 5-6, 1998 one-year following herbicide application to access the damage. Plants tagged in 1997 were relocated, measured and inspected for damage. The surrounding area where large populations were found in 1997 was searched again for *Botrychium* in 1998.

Two formal plots (1 and 2) were established in 1997. A third site (referred to as plot 0) represents 22 plants marked in a $2m^2$ area that I was interested in following because of their size and potential to be *B. gallicomontantum*. (A formal plot was not placed at this site due to time constraints.)

A wildfire (started accidentally) burned the northern portion of the prairie in early April 1998. It was evident that many plants had emerged prior to the burn and bore singed leaf margins. Some plants were more severely damaged and were brown and wilted. There were significantly fewer plants in 1998 than in 1997. In 1997 the prairie contained 1000's of plants whereas in 1998 the plants were scattered and fewer (estimated to be less than 500). This may be due to three possible factors. First the plants may have not returned because of herbicide damage in 1997. Second they may have been damaged because of fire in 1998. Third, it is normal for moonwort populations to fluctuate widely without any apparent cause. More years of data collection will help to resolve which factor is the likely cause of the decrease.

Two species of *Botrychium* were discovered at Lake Bronson in 1997, *Botrychium gallicomontanum* and *B. simplex*. Because of the herbicide damage in 1997 and fire damage in 1998 it was difficult to identify damaged plants and consequently the population size of *Botrychium gallicomontanum* could not be determined. It appeared that *B. simplex* was more abundant than *B. gallicomontanum*. However, a year in which there is no damage (herbicide or fire) to plants is necessary to determine the population size and species balance.

Similar difficulties (identification and quantification) were encountered monitoring the plots. There is a notable decrease in leaf size and population size in 1998 (Table 1.) Plot 1 was not burned and plots 0 and 2 were burned. In 1998 the population declined 33% in plot 1 and 23% and 57% in plots 0 and 2, respectively. Leaf size also decreased with the lowest decrease in plot 1 (14% smaller) followed by 60% and 31% decrease in plots 0 and 2 respectively.

Table 1. Comparison of all plants (sprayed and healthy).

Plot - Year	0 - 1997	0 - 1998	1 - 1997	1 - 1998	2 - 1997	2 - 1998
# of Plants	22	17	60	45	86	37
Ave. Tropophore Size (cm)	4.9	2.0	2.1	1.8	2.4	1.6
Std. Deviation	2.4	1.7	1.2	1.1	1.4	1.3

Because of the herbicide and burn history of these plots caution must be exercised in drawing conclusions from this data. Additional monitoring is necessary to determine the effect of the herbicide and fire on these populations. Field observations revealed an erratic pattern of herbicide dispersal, such that some areas (and consequently some *Botrychium*) were hit hard and other areas were not hit at all. It appears that plants which were a "direct herbicide hit" did not return, but that many plants did return which were perhaps missed or hit lightly by the herbicide.

It is possible to compare those plants which sustained visible herbicide damage in 1997 and reappeared in 1998 (Table 2) with plants without apparent herbicide damage in 1997 (Table 3). Leaves hit by herbicide show a decrease in population and leaf size. From this preliminary data it appears that plants hit by herbicide were smaller the subsequent year than those without herbicide damage.

Table 2. Comparison of plants which had obvious herbicide damage in 1997.

Plot - Year	0 - 1997	0 - 1998	1 - 1997	1 - 1998	2 - 1997	2 – 1998
# of Plants	4	3	42	11	77	21
Ave. Tropophore Size (cm)	2.3	0.8	2.2	2.0	2.5	1.7
Std. Deviation	0.8	0.3	1.2	1.4	1.5	1.2

Table 3. Comparison of plants which were healthy with no herbicide damage in 1997.

Plot - Year	0 - 1997	0 - 1998	1 - 1997	1 - 1998	2 - 1997	2 – 1998
# of Plants	17	5	16	38	7	28
Ave. Tropophore Size (cm)	5.7	2.5	1.9	2.0	1.4	1.5
Std. Deviation	2.2	1.3	1.2	0.9	0.8	1.2

In 1998 there were 21 and 15 new plants in plots 1 and 2 that had not been present in 1997. Plants which were underground either as juvenile sporophytes which have not yet emerged or as dormant adult sporophytes at the time of herbicide application probably were not affected by the herbicide. These "new" recruits are typical of moonwort populations and might sustain the population despite one year of herbicide application.

Moonwort populations are highly buffered due to the underground portion of the lifecycle. Underground gametophytes and developing sporophytes may allow the population to rebound from relatively infrequent "bad" years. However the number of spores released in 1997 was probably significantly smaller because of the effects of herbicide. Presumably this will affect the population several years hence when this reduced cohort of spores filters down through the soil, germinates and eventually produces emergent sporophytes, a process that may take five years or more (Johnson-Groh, Farrar and Miller 1998). It is thus important to emphasize that the lasting effect of the herbicide through a reduced spore set may not be detected for another five years.

While it is still too early to determine the long-term affects of herbicide application I anticipate that there will be a small decline in the population. More years of data will help sort out the effect of herbicide from fire and normal annual population variation.

No studies have been conducted on the long-term effects of herbicide on *Botrychium*. This "incidental" experiment will provide important data on which to base management practice.

***Botrychium mormo* – Chippewa National Forest and Superior National Forest**

B. mormo is a maple-basswood forest species that also has a limited distribution but has been found increasingly with searches. *B. mormo* is rare in Wisconsin and Michigan with the highest density of sites found in the Chippewa National Forest, Minnesota. Based on recent allozyme evidence *B. mormo* is very closely related to a western species, *B. montanum* (D. Farrar, pers. comm.). It is likely that these two taxa may be varieties of one species with a primary distribution centered in the western states and peripheral populations in the Great Lakes States.

Five permanent monitoring plots for *B. mormo* have been established in northern Minnesota. Three sites are located in the Chippewa National Forest. Two plots on the Ottertail Peninsula include a mature maple-basswood stand monitored since 1992 and a younger maple-basswood stand monitored since 1994. A third plot located in the Meadow Lake District of the Chippewa National Forest is in a maple-basswood forest with scattered aspen monitored since 1994. The easternmost *B. mormo* plot is in the Pike Mountain area of Superior National Forest and the westernmost plot is in the Hagen Wildlife Protection Area in Polk County.

The overall average number of plants per plot peaked in 1996 with an average of 98.6 plants per plot (Figure 4). The lowest population totals have occurred recently with an average of 19 plants per plot in 1998. Similar to the prairie moonworts the overall trend is down, with plots varying independently. Some populations have decreased precipitously in the last two years. This decrease has probably been caused by several factors including drought, introduced worms and natural population fluctuation.

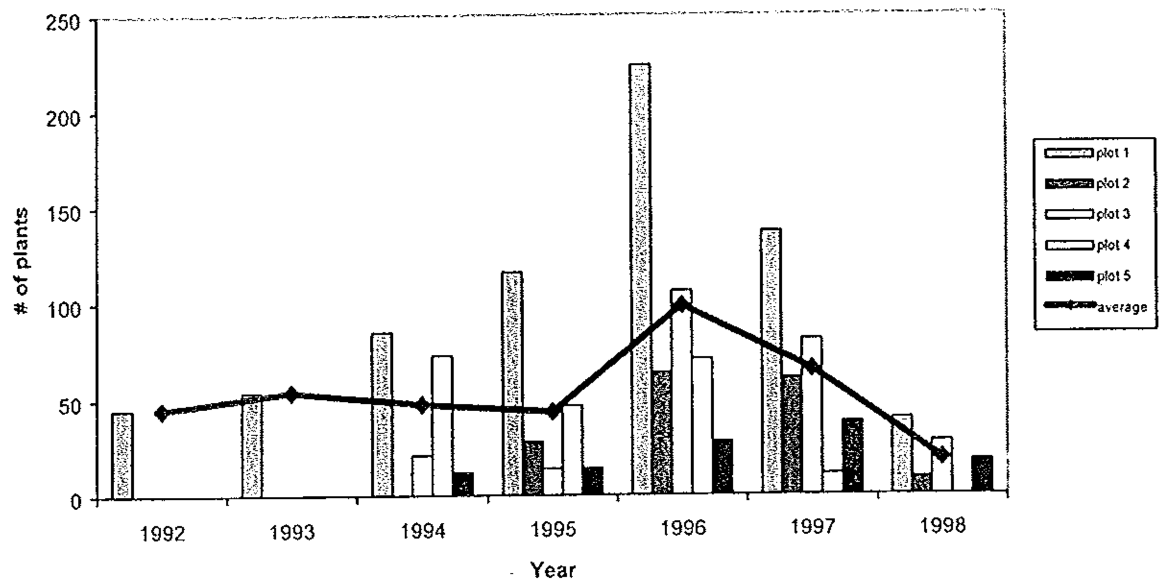

Figure 4. Population trends for *B. mormo* plots in northern Minnesota.

A significant concern in the Chippewa National Forest is the impact of introduced earthworms on the ecosystem and specifically on plants such as *Botrychium* which are mycorrhizal. It is quite probable that earthworms may substantially alter the soil enough to reduce and possibly eliminate *Botrychium*. The concurrent loss of duff with worm invasion is alarming and undoubtedly affects the mycorrhizae on which *Botrychium* is dependent. It seems unlikely that worms eat *Botrychium* and informal experiments in the lab of Johnson-Groh have corroborated this observation.

Worms have affected two plots; both are located on the Ottertail Penninsula of Chippewa National Forest. The soil was screened for worms in the vicinity of plot 4 in 1997 and high densities of introduced worms were discovered. The population of plot 4 decreased in 1997 to 11 plants and none were present in 1998. Though it seems likely that worm invasion has fatally affected the population, it is important to note that all populations decreased in 1997 and 1998. Conclusions regarding affects of worms must be made cautiously. Plot 1 was accessed for worms in 1997 and found to be "worm-free", but it also exhibited a significant decrease from a population high in 1996. Evidence (lack of duff) in plot 1 in 1998 indicates recent worm invasion which concurred with another significant population decrease.

It appears that worm invasion has had a significant initial impact on some populations. However other factors must also be noted as all populations decreased but not all populations have been impacted by worms. First, 1998 was a very dry summer and the soil was extremely dry when the populations were monitored. Dry conditions would reduce the mychorrizal resources available to *Botrychium*. Second, it is normal for moonwort populations to fluctuate widely without any apparent cause (see 12 years of data for prairie moonworts). Third it is plausible that *Botrychium* population crashes following years of high population are a regulating device. In short the population has exceeded the carrying capacity of that particular site and the population crashes. (The limiting resource is probably mychorrizae.)

More years of data collection will help to resolve which factor is the likely cause of the decrease. Even if exotic worms impact *Botrychium* populations it remains to be seen whether the impact is lasting or whether populations will rebound. Other species of *Botrychium* have demonstrated great resiliency *(B. gallicomontantum* following fire and drought). Because of the prolonged period of time this plant spends underground it is impossible to access the impact following one or two growing seasons. It is possible for worms (or some other cause) to essentially eliminate all sporophytes above and below ground. Because the spores still remain in the soil, it is likely that they will regenerate the population, however this may take years (as is the case for *B. gallicomontanum* following fire). It also must be noted that the population will only rebound if suitable resources (mycorrhizae, moisture, etc.) are available for the reestablishment of the population.

Related Studies

Permanent Plot Monitoring Studies – Iowa, Oregon, Alaska

Similar long-term ecological monitoring of *Botrychium* has been conducted in Iowa, Oregon and Alaska. Iowa studies have been conducted since 1987. Western plots have been established recently (1997-98) primarily to access impact of management (fire and timber harvest) on *Botrychium*. These management studies will provide useful data for comparisons with Minnesota species.

Similar population trends have been found in Iowa for *Botrychium campestre* which is found primarily in the western portion of Iowa in the loess hill prairies. We have 12 plots established in a large Nature Conservancy Preserve, 5-Ridge Prairie. Monitoring began in 1987 with the establishment of three plots in different burn units. A trend similar to what is seen in Minnesota is evident in these Iowa plots with populations declining in recent years (Figure 5). The overall average number of plants per plot peaked in 1991 with an average of 27 plants per plot. The lowest populations have occurred recently with an average of 4 plants per plot in 1998.

Phenology

Temporal and demographic aspects of the growth of *Botrychium gallicomontanum* and *B. mormo* leaves were studied in 1996 and 1997 (Johnson-Groh and Lee, 1998). A total of 219 *B. gallicomontanum* plants were monitored at Frenchman's Bluff and a total of 412 *B. mormo* plants were monitored at two sites in the Chippewa National Forest. Events during growth were divided into four stages including emergence, leaf separation, spore release and senescence. *B. gallicomontanum* began emerging in April, peaked in the first week in June and declined rapidly following this peak. Though the population size declined in late June, the largest plants were found in late June and early July with an average size of 4.8 cm. *B. mormo* plants emerged in June and the population size peaked in early July, considerably earlier than previously thought. The largest plants occurred late in August with an average size of 2.7 cm. The average "seasonspan" or period of existence aboveground, for *B. gallicomontanum* and *B. mormo* respectively was 5.5 wks and 8.5 wks. Late emerging plants produce spores in a shorter period. The emergence stage is prolonged in *B. gallicomontanum* plants, whereas *B. mormo* plants have a much longer separation stage. Understanding the phenology will help us to more accurately predict the impact of management practices such as fire or timber harvest on these rare species.

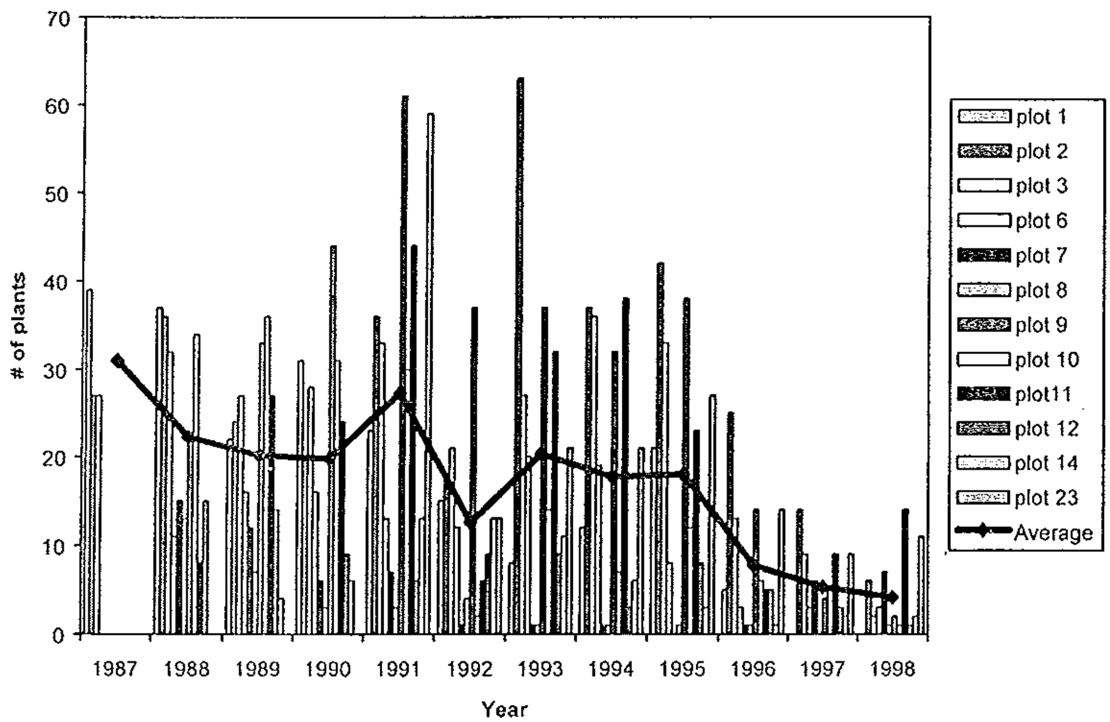

Figure 5. Population trends for *B. campestre* plots at 5-Ridge Prairie, Plymouth County, Iowa.

Herbivory

From studies thus far we know that moonworts are mycorrhizal and sporadic in appearance above ground and that individual plants typically skip years, not emerging annually. This lack of dependence on annual photosynthesis along with the occurrence of albino plants indicates that moonworts probably derive most of their sugars from other nearby species via a mycorrhizal connection; that is they are more or less parasitic on the mycorrhizal system. Removing leaves harms the plants ability to disperse spores, but probably has little effect on their overall carbon balance. Plants that have lost leaves should be equally likely to reemerge the following year as plants that are not impacted. Herbivory is a form of leaf removal that we have monitored through permanent plots. It appears that this type of removal of the leaf has little or no impact (Johnson-Groh and Farrar, 1996). However, herbivory (above and below ground) is spatially and temporally patchy, and the extent of damage varies considerably.

Fire

Fire has the same affect as herbivory in the removal of photosynthetic lamina. Observations on the effects of fire have been conducted in Iowa and Minnesota (Hole-in-the-Mountain, Lincoln County, MN) on *B. campestre*. We have compared populations in plots burned in the fall, spring or not at all. It appears from this data that burning itself may not be damaging to *Botrychium*. However, fire combined with excessive drought or erosion (both natural results of fire) can be harmful to *B. campestre* (Johnson-Groh and Farrar, 1995; 1996). These studies included plots which were burned as part of the ongoing preserve management. Often the burns were incomplete leaving plots only partially burned. However because of the imprecise control of burns, a low density of plants in relatively few plots and site differences between burned and unburned this data has been difficult to interpret.

Distribution and Abundance of Underground Gametophytes and Juvenile Sporophytes

A significant portion of the moonwort's life history, the gametophyte and juvenile sporophytes stages, are spent underground. We have conducted experiments to determine the distribution and abundance of the underground gametophytes and sporophytes of *B. campestre* in Iowa, *B. gallicomontanum* in Minnesota and *B. mormo* in Minnesota. Soil samples were collected in a systematic spoke-like design using a bulb-digger and then sieved through a series of soil sieves to extract the mineral soil and large organic material. These samples were then processed using a centrifugation technique (Mason and Farrar, 1989) that allows the lighter plant material to be collected for examination under the microscope.

Results vary greatly between species (Table 4) and are preliminary. *B. campestre and B. gallicomontanum* both produce gemmae but vary considerably in their general abundance. The gametophytes of *B. mormo* are extremely abundant and appear to behave differently, than other species. Because *B. virginianum* is a very common species it was hypothesized that the underground stages would also be abundant. In an area of 201 m^2 containing 114 plants, 49-0.5cm diameter samples were sieved. From these only 7 gametophytes were extracted indicating a gametophyte density of approximately 70/m^2 and a total of more than 14,000 in the population area. This project is ongoing and includes several additional western species currently under investigation in Johnson-Groh's lab.

Table 4. Abundance of underground structures for four *Botrychium* species.

Species	Ave # gametophytes/ m^2	Ave # sporophytes/m^2	Ave # gemmae/m^2
B. campestre	21	180	6,023
B. gallicomontanum	10	10	4000
B. mormo	700	250	No gemmae
B. virginianum	70	-	No gemmae

SUMMARY

Botrychium are fascinating and elusive plants. Despite 12 years of monitoring populations we still understand only portions of the life history. Most notably the underground portion of the life cycle remains mysterious and our inability to cultivate *Botrychium* complicates learning about this portion of the life cycle.

We have learned several things from our *Botrychium* studies:

1. Moonworts are mycorrhizal and sporadic in appearance above ground. Individual plants typically skip years and do no emerge annually. It is not uncommon for species to skip up to 3 or 4 years and still reemerge. (This varies depending on the species.)
2. Populations fluctuate independently among plots in any given sites. Some populations may be increasing while others are decreasing. These differences reflect microsite differences such as soil moisture, herbivory, or mycorrhizae. Each of these populations varies independently within a metapopulation and some may be declining and dying out while others are thriving.
3. Monitored populations of both *B. campestre* and *B. mormo* have exhibited overall declining numbers. Possibly these trends indicate finite life spans of individual populations. Given the great variability we have observed among plots, it is quite possible that elsewhere populations are building.
4. Fire, herbivory, herbicide and timber harvest (studies on western species) have an immediate impact on the aboveground sporophytes. The long-term effect of these factors on belowground structures (gametophytes and juvenile sporophytes) is still unknown. More years of monitoring are needed given the length of time it takes for *Botrychium* to develop (perhaps 5 to 7 years from spore release to sporophyte emergence).
5. *Botrychium* are dependent on mycorrhizal resources and their behavior is a function of the availability of this resource. Repeated removal of leaf tissue may be inconsequential to the plant's ability to survive. However reductions in water resources which presumably affect mycorrhizal resources have significant impacts on the populations. Mycorrhizae are the most limiting factor for *Botrychium* establishment, distribution and abundance.

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entire pteridophyte floras will 1) relate patterns of distribution of rare/endemic taxa to the pteridophyte life cycle, 2) describe efforts of propagating and reintroducing endangered species, 3) explain the construction and use of pteridophyte biodiversity databases, and 4) document efforts to incorporate local communities in regional conservation efforts.

273 BENNERT, H. WILFRIED. Spezielle Botanik, Ruhr-Universitaet, D-44780 Bochum, Germany.—*Risk analysis for pteridophytes in Germany.*

When analyzing endangered plant species and developing population-orientated conservation strategies, information on distribution, status of populations (e.g., declining or stable) and biological risk (like poor reproduction, stenotopic behaviour) is required. We report on such a database assembled for a group of endangered and/or rare German pteridophyte species. A total of 20 species was investigated between 1992 and 1996 by completely evaluating all extant populations. Using these results, the species were arranged in 5 groups depending on number and size as well as on status of populations.

274 HOOT, SARA B.*, AMY B. KORNKVEN, AND W. CARL TAYLOR. Department of Biological Sciences, University of Wisconsin, Milwaukee, WI 53201 and Botany Section, Milwaukee Public Museum, Milwaukee, WI 53233.—*The use of amplified fragment length polymorphisms (AFLP) in assessing genetic variation in the endangered species, Isoëtes louisianensis*

The use of molecular techniques is increasingly important in the conservation biology of endangered species as a means of understanding the genetic structure and diversity present in populations. Recent developments in molecular techniques include a powerful new tool for generating DNA fingerprints and detecting genetic polymorphisms: amplified fragment length polymorphisms (AFLP). This technique uses PCR to amplify a random assortment of DNA restriction fragments generated using two enzymes. AFLP technology combines the reliability of restriction digestion of genomic DNA with the benefits of PCR-based assays by ligating primer recognition sequences, or adaptors, to the restricted DNA. Because of the amplification step, only a small amount of DNA is needed. The technique allows the characterization of a large number of genetic loci in a relatively short time frame and is reliable and reproducible. We present preliminary results of the application of AFLP technology to the endangered species, Isoëtes louisianensis (Louisiana quillwort), currently known in two parishes of southeastern Louisiana and eight counties in southern Mississippi. Genetic diversity appears to be low in many of the populations, especially those with small numbers of individuals. There appears to be a positive correlation between geographical distance and genetic distance. In a preliminary neighbor joining analysis based on three primer pairs (61 variable characters, 25 informative characters), individuals from the same populations and geographical locations cluster together at high bootstrap values.

275 JOHNSON-GROH, CINDY, L*, DONALD, R. FARRAR, AND PHILIP MILLER. Biology Department, Gustavus Adolphus College, St. Peter, MN 56082; Botany Department, Iowa State University, Ames, IA 50011; Conservation Breeding Specialist Group, Apple Valley, MN 55124.—*Modeling extinction probabilities for moonwort (Botrychium) populations.*

Several Botrychium species are classified as endangered or threatened. Rare species like B. gallicomontanum and B. pseudopinnatum, have only a few known populations. Conservation management of these rare Botrychium requires knowledge of their life history and population demographics. Using what we know about the species biology we can model population responses to natural and man-made perturbations. Understanding Botrychium population dynamics, (the distribution and actual population size) is complicated by their small size. It is further compounded by the fact that plants do not produce above-ground leaves on an annual basis and may be dormant for several years. High variability in numbers of above-ground plants suggests a high probability of local extinction. Using demographic data collected on Botrychium mormo we have identified the critical life history stages and used a stage-based model to assess the population viability. Stages identified for Botrychium include above-ground stages and several underground stages including spores, gametophytes, juvenile sporophytes and non-emergent sporophytes. Stochastic fluctuations and catastrophic disturbances were examined. The model resulting from this analysis predicts greater stability of populations than might be concluded from monitoring above-ground plants. We believe this is a consequence of having a large proportion of the population existing in underground stages. Even catastrophic elimination of all the underground gametophyte and sporophyte stages does not inevitably lead to population extinction because of the importance of the spore bank. Despite highly variable above-ground population fluctuations, below-ground stages provide Botrychium populations with a high degree of buffering against local extinction.

PTERIDOLOGICAL SECTION

POSTERS

376 AMOROSO, CECILIA B.*, VICTOR B. AMOROSO, AND VICTOR O. GUIPITACIO. Department of Biology, College of Arts and Sciences, Central Mindanao University, 8710 Musuan, Bukidnon, Philippines. - In vitro culture of *Cyathea contaminans* (Hook.) Copel.

Cyathea contaminans (Hook.) Copel. is commonly known as tree fern in the Philippines. The tree ferns grow naturally in the forests of Bukidnon, Mindanao, Philippines. They have multiple uses as ornamentals and their trunks are used as house posts and for potting orchids. Continuous harvesting of the trunks of *C. contaminans* without replanting may result in the gradual disappearance of this species from the forests. Thus, there is a need to find ways of conserving this fern. This study was conducted to determine gametophyte development of *C. contaminans* through in vitro culture. The spores of *C. contaminans* were inoculated in spore culture medium and these germinated 11-12 days after sowing. The pattern of spore development was of the *Cyathea* type while prothallial development was of the Adiantum type.

377 JOHNSON-GROH, CINDY L.* AND DONALD R. FARRAR. Department of Biology, Gustavus Adolphus College, St. Peter, MN 56082 and Department of Botany, Iowa State University, Ames, IA 50011. - The effects of fire on prairie moonworts (*Botrychium* subgenus *Botrychium*).

Populations of three species of moonwort ferns in native prairies of western Iowa and western Minnesota have been monitored since 1987 with a permanent tag marking the site of each plant. Presence/absence, size and evidence of injury (fire or herbivory) for each plant have been recorded annually through 1996. The unusual biology of prairie moonworts, including their mycorrhizal relationship, allows these plants to not emerge in some years and reappear in subsequent years, making it difficult to assess the effects of fire. On average, less than half of the known plants in a population produce above-ground leaves in a given year. *Botrychium campestre*, *B. gallicomontanum* and *B. simplex* show similar patterns of occurrence and response to fire, drought and other environmental disturbances. The effects of fire on *B. gallicomontanum* are of particular concern because it is endemic to a single site in western Minnesota. The total number of recorded plants (those producing above-ground leaves) and plant size show no significant difference between burned and unburned sites in most years. However, fires occurring during or after a drought have resulted in population decline. Slow recovery from fire, compounded by drought, suggests that a substantial number of plants are killed by this combination.

SYSTEMATICS SECTION

CONTRIBUTED PAPERS

378 ABDEL MIGID, HALA* and RICHARD G. OLMSTEAD. Department of Botany, Box 355325, University of Washington, Seattle, WA 98195. - Molecular Systematics of tribe Hyoscyameae (Solanaceae).

The Solanaceae achieve their greatest diversity in the Western Hemisphere. There are several groups within the family that have disjunct distributions that include New and Old World members, however there are very few groups whose distribution is restricted to the Old World. Tribe Hyoscyameae comprises seven genera and is the most diverse group in the family that is exclusively Old World, outside of Australia. The greatest generic diversity occurs in China with five genera represented, two of which are endemic. A traditional characteristic of the tribe, the circumscissille dehiscence of the fruit, excludes two other Old World genera with similar vegetative morphology but berry-like fruits, Atropa and Mandragora. Chloroplast DNA data indicate that Atropa, formerly placed in tribe Solaneae, belongs in Hyoscyameae, but that Mandragora (monogeneric Old World tribe Mandragoreae) apparently is not closely related. In previous studies within the family, PCR and direct DNA sequencing have enabled the systematic study at different taxonomic levels. This approach provides a good tool for phylogenetic inferrence among genera within Hyoscyameae as well as between Hyoscyameae, Mandragoreae and other tribal relationships.